The In vitro Osteoclast Differentiation in Arthritis (IODA) project: Osteoclastogenesis as a marker of presence and activity of disease in Arthritis.

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Introduction

Osteoclasts play an important role in the pathophysiology of rheumatoid arthritis (RA) and osteoarthritis (OA). We hypothesize that *in vitro* osteoclastogenesis and the activity of these osteoclasts correlate with disease presence in RA and OA patients and with disease activity in RA. We previously showed that patients with RA have higher bone resorption rates and that patients with inactive RA have a higher osteoclastogenesis rate than the group with active RA or controls. In this study we analyzed the OA cohort.

Objectives

1. To determine whether osteoclast function, osteoclastogenesis, and other related factors are related to the presence of OA (by contrasting OA patients and controls).
2. To find diagnostic markers for OA and to use these markers to build a strong diagnostic model.

Methods

Subjects

We selected 89 patients with OA and 35 self-reported healthy controls among the patients who were recruited from the outpatient clinic at the Division of Rheumatology, Centre Hospitalier Universitaire de Sherbrooke. The selection included removal of patients/controls with missing information and balancing of the age of the members of the two populations.

Osteoclastogenesis

Peripheral blood mononuclear cells were separated from blood by Ficoll gradient and the number of CD14+ cells was determined by flow cytometry. The cells were cultured in the presence of RANKL and M-CSF and the number of OCs was evaluated after 21 days. Bone resorption was quantified on cortical bone slices stained with toluidine blue. OC apoptosis was evaluated by colorimetric assay (TACSTM TdT Blue Label kit).

Data analysis

We performed classical statistical tests to measure significance of differences between the two patients groups. Several markers related to osteoclastogenesis related factors were found, see Figure 1.

We investigated pairs of the discovered markers and found that certain combinations lead to improved predictive quality when compared with using individual markers, see the upper panel in Figure 2.

We also used modern machine-learning algorithms to develop a human-readable diagnostic model in the form of *alternating decision tree*. This model is relatively easy to comprehend as opposed to other mathematical or probabilistic models. It provides better predictive quality when compared with individual or paired markers, and gives insights into the diagnostic procedure, see Figures 3 and 4. The decision tree model is represented in 3D space in the lower panel of Figure 2.

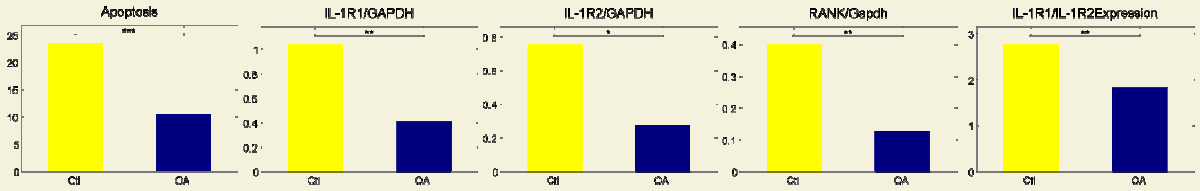

Figure 1. Statistical analysis showing the difference in mean values between the OA and control (Ctl) groups for the statistically significant markers.

* P value < 0.05, ** P value < 0.01, *** P value < 0.001; T-test and Mann-Whitney test were used for normal and non-parametric variables, respectively; Shapiro-Wilk test was used to verify normality.

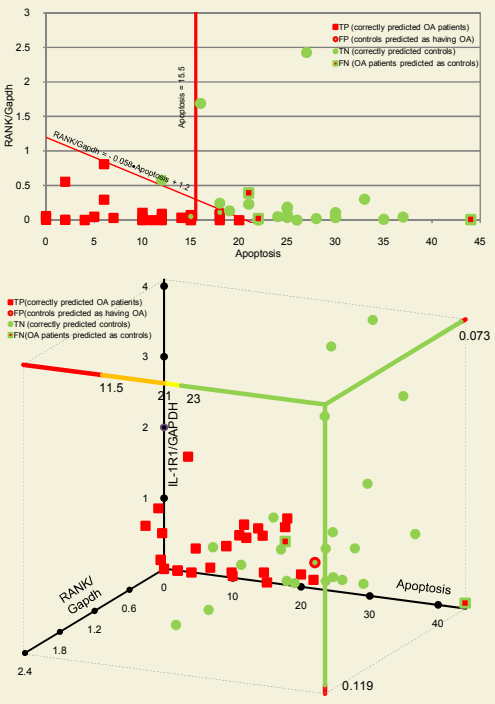

Figure 2. The upper panel presents two dimensional scatter plot for Apoptosis and RANK/Gapdh, which includes 1D (for Apoptosis) and 2D diagnostic models denoted by red lines that separate positive and negative samples. The lower panel shows three dimensional scatter plot for Apoptosis, RANK/Gapdh and IL-1R1/GAPDH, which visualizes the diagnostic model from Figure 3. The outer axes are annotated with color where green corresponds to controls and red corresponds to OA patients.

TP and FN represent the OA patients correctly predicted as having OA and incorrectly predicted as controls, respectively. TN and FP represent controls correctly predicted as disease free and incorrectly predicted as having OA, respectively. The plots (and below accuracy measurements) include data for 49 patients who have values of all three markers available.

accuracy for 1D model (Apoptosis = 15.5) equals 83.7 %

accuracy for 2D model (RANK/Gapdh = -0.058 Apoptosis + 1.2) equals 89.8%.

accuracy for 3D model (representation of the alternating decision tree from Figure 3 based on Apoptosis, RANK/Gapdh and IL-1R1/GAPDH) equals 93.9%.

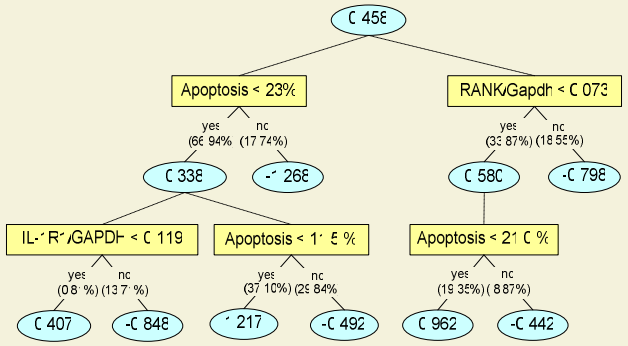

Figure 3. Alternating decision tree model for diagnosis of OA.

Descending from the top node downwards along the branches, if the sum of scores (numbers in blue ovals) satisfying the conditions (shown in associated yellow rectangles) is positive then OA is present. Otherwise (negative sum), the subject is assumed not to have OA. The bigger the sum (in either direction), the stronger the confidence associated with the prediction/diagnosis. Values in parentheses (below "yes" and "no") express the percentages of patients and controls satisfying (or not) a given condition.

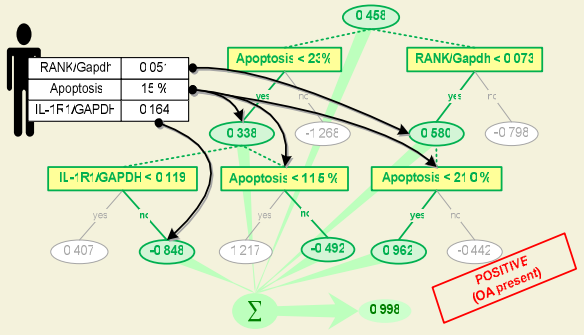

Figure 4. Example application of the diagnostic model from Figure 3.

The osteoclastogenesis characteristics, measured for an example patient, are compared against the model in Figure 3. The sum of the branches satisfying the conditions is positive, which suggests that the patient has OA.

Results

The analysis revealed the following:
1. cells from patients with OA had lower apoptosis rates than controls;
2. protein expression of RANK and IL-1 receptor 1 and 2 was higher in controls than in OA patients; and
3. low apoptosis combined with low RANK/GAPDH values were associated with OA.

The diagnostic decision tree model was built using information concerning demographics, clinical observations, and osteoclastogenic measurements. The model shows that only three markers are sufficient to provide high quality predictive performance.

Accuracy is the fraction of the correctly classified patients among all patients.
Accuracy = (*TP*+*TN*) / (*TP*+*FP*+*TN*+*FN*)

Sensitivity is the number of correctly predicted OA patients among all OA patients.
Sensitivity = (*TP*) / (*TP*+*FN*)

Specificity is the number of correctly predicted controls among all controls.
Specificity = (*TN*) / (*FP*+*TN*)

	Accuracy	Sensitivity	Specificity
Cross validation	87.9%	92.1%	77.1%
Self consistency	90.3%	93.3%	82.9%

Cross validation quantifies the ability of a model to classify unseen data (we performed 10 fold cross validation), while self consistency shows how well the model describes the data it was built from.

Conclusions

These results indicate that parameters important to osteoclast biology correlate with the presence of OA and that they can be used to build a high quality diagnostic model.

Acknowledgement

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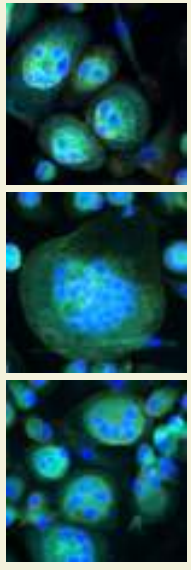

Immunofluorescent images which were used to assess OC multinucleation. Images were taken on a Zeiss epifluorescent microscope. Cells were fixed with paraformaldehyde and stained for cytoskeleton (cyan) and nuclei (blue). The nuclei per cell were quantified from 20 random microscope fields of view per patient.